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THE EFFECT OF INHALED NITRIC OXIDE ON SMOKE INHALATION INJURY IN AN OVINE MODEL

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Smoke inhalation is a significant comorbid factor in thermal trauma. The effect of inhaled nitric oxide (NO) on smoke inhalation injury was evaluated in an ovine model. Following smoke exposure, group 1 animals (n = 9) spontaneously breathed room air, and group 2 animals (n = 8) breathed 20 parts per million of NO in air for 48 hours. Cardiopulmonary variables and blood gases were serially measured; bronchoalveolar lavage (BAL) was performed and wet-to-dry lung weight ratios (W/D) determined at 48 hours. Pulmonary vasoconstriction following smoke inhalation was significantly attenuated by inhaled NO ($p < 0.05$), which exerted no apparent effect on the systemic circulation. In group 2, the serial decline in pulmonary oxygenation was less than in group 1, consistent with a smaller physiologic shunt ($p < 0.05$). There were no significant differences in W/D, lung compliance, BAL fluid analysis results, or histologic evaluation findings between the two groups. These results suggest that inhaled NO exerted beneficial effects on pulmonary arterial hypertension and oxygenation following smoke inhalation without apparent amelioration of airway inflammation.

SMOKE INHALATION causes acute airway inflammation and subsequent pulmonary edema that are partially mediated by activated leukocytes and various inflammatory cytokines.[1] These changes impair pulmonary function by increasing ventilation-perfusion mismatching and ultimately enhancing susceptibility to pulmonary infection, which increases both morbidity and mortality in patients with thermal injury.[2,3] Medical interventions that attenuate this deleterious process may exert a beneficial effect on outcome.

Nitric oxide, an endothelial-derived relaxing factor, is generated from L-arginine in mammalian tissues.[4] Nitric oxide is a lipophilic free radical with a short half life, which appears to act through a signal transduction event on local cells.[5] Nitric oxide activates the synthesis of intracellular guanosine 3′,5′-cyclic monophosphate (cGMP) and reduces intracellular free calcium concentration, leading to smooth muscle relaxation as well as inhibition of platelet aggregation and adhesion.[6,7] In addition, nitric oxide has the potential to modulate neurotransmission, cell mitogenesis, immune function, and inflammation induced by cytokines.[4,5,8]

Inhaled nitric oxide (5–150 ppm) has been recently reported to act as a selective pulmonary vasodilator without causing systemic vasodilation. Pulmonary vasodilatation following inhalation of nitric oxide has been demonstrated in animal models of pulmonary arterial hypertension induced by a thromboxane analog, heparin-protamine treatment, hypoxemia, and sepsis.[9–11] Beneficial effects of inhaled nitric oxide have been reported in patients with pulmonary hypertension, adult respiratory distress syndrome (ARDS), congenital heart failure, chronic obstructive lung disease, and pneumonia.[12–16]

To our knowledge, the effect of inhaled nitric oxide on chemically induced lung injury has not been examined. This study evaluated the effects of inhaled nitric oxide on smoke inhalation injury in an ovine model.

MATERIALS AND METHODS

Animals and Preparations

Twenty-four random-source male sheep 1 to 2 years old, weighing 25 to 32 kg, were used in this study. The animals were housed in covered outdoor runs, treated for parasites (ivermectin, 0.2 mg/kg, IM) and fed commercial chow and water ad libitum. The animals were divided into three groups. Group 1 (n = 9) breathed room air following smoke exposure. Group 2 (n = 8) breathed 20 parts per million (ppm) nitric oxide in air following smoke exposure. Group 3 (n = 7) was used to establish normal values for bronchoalveolar lavage studies and wet-to-dry lung weight ratios. All

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study protocols were approved by the U.S. Army Institute of Surgical Research Animal Use Committee and adhered to the provisions of the Animal Welfare Act.

On the day before smoke exposure, the animals in groups 1 and 2 were instrumented while anesthetized with sodium pentobarbital (25 mg/kg, IV, Sigma Chemical Co., St. Louis, Mo.). Silicone rubber cannulae were placed in a femoral artery and vein. One radiopaque sheath introducer, through which a Swan-Ganz catheter was placed, was inserted into an external jugular vein.

Smoke Exposure Methods

After 24 hours, the animals in groups 1 and 2 were anesthetized with sodium pentobarbital, and a tracheostomy was performed using a 9.0-mm tracheal tube. The animals were paralyzed with succinylcholine chloride (0.5 mg/kg IV, Abbott Laboratories) before smoke exposure.

Smoke was generated by thermolysis of pine woodchips (60 g) in a crucible furnace (Furnace Model 56622 and Control Console Model 58114, Lindberg, Watertown, Wis.) at a constant temperature of 400°C and an air flow of 6.0 L/minute. Smoke was delivered into a 20-L reservoir and mixed with a 2.0-L/minute flow of 100% oxygen. Animals received nine exposure units of this mixture; one exposure unit consisted of five breaths (tidal volume 30 mL/kg, with a breathhold of 5 seconds) and a 5-second rest between exposure units.

Following smoke exposure, the animals in groups 1 and 2 were housed in individual cages in a climate-controlled facility, and observed for 48 hours in the awake state while breathing spontaneously. The animals received a maintenance intravenous infusion of lactated Ringer's solution (1.5 mL/kg/h).

Inhalation of Nitric Oxide

Thirty minutes following smoke exposure, the tracheal tube of each animal was connected to a nonrebreathing circuit consisting of a 5-L reservoir bag and one-way valves to separate inspired from expired gas. The inspired gas was room air (F_{IO_2} 0.21) for group 1. Group 2 breathed a mixture of room air and 100% oxygen that was diluted with nitric oxide (237 ppm in N_2, Air Product Co., Austin, Tx) to an inspired concentration of 20 ppm nitric oxide, with an F_{IO_2} of 0.21. The inspired gas flow was 20 L/minute and the expired gas was discarded.

Measurements

Oxygen, nitric oxide, and nitrogen dioxide concentrations of the inspired gas were continuously measured with gas monitors (Models P2138, P2170, P2160, Conspec). Cardiopulmonary variables and blood gas levels were measured before smoke exposure and at 1, 3, 6, 12, 24, 36, and 48 hours following exposure. Pulmonary artery pressure, pulmonary capillary wedge pressure (PCWP), and systemic artery pressure were measured using a pressure monitor (Model 78354A, Hewlett-Packard Company). Cardiac output was measured by the thermodilution technique (Cardiac Output Computer Model 9520A, American Edwards Laboratory). Blood gas analyses were performed using an IL 1303 pH/blood gas analyzer and an IL 282 Co-oximeter (Instrumentation Laboratories, Inc.). Plasma samples for the measurement of conjugated dienes and nitrate were stored at −70°C until measurement.

Respiratory index (RI), an oxygenation capacity index, and physiologic pulmonary shunt (Qs/Qt) were calculated by the following formulae

$$RI = (P_{AO_2} - Pa_{O_2})/Pa_{O_2}$$

where P_{AO_2} is alveolar O_2 pressure (mm Hg); and Pa_{O_2} is arterial O_2 pressure (mm Hg) and

$$Qs/Qt(\%) = 100 \times (Cc_{O_2} - Ca_{O_2})/(Cc_{O_2} - Cv_{O_2})$$

where Cc_{O_2} is pulmonary capillary O_2 content (mL/dL); Ca_{O_2} is arterial O_2 content (mL/dL); and Cv_{O_2} is mixed venous O_2 content (mL/dL).

Static lung compliance and pulmonary resistance were measured before smoke exposure and 48 hours following smoke exposure while the animals were being mechanically ventilated. Using an esophageal balloon, transpulmonary pressure was measured with a differential transducer (MP-451, Validine Engineering Corp., Northbridge, Calif). Inspiratory tidal volume was measured with a Wright spirometer. Air flow rate at the external orifice of the tracheal tube was measured with a pneumotachograph (Model 17212, Gould, Inc., The Netherlands). Static lung compliance was calculated by dividing the tidal volume by the transpulmonary pressure difference between the plateaued end-inspiratory-pause phase and the end-expiratory-pause phase. Pulmonary resistance was calculated by dividing the inspiratory transpulmonary pressure change by the inspiratory air flow rate.

Forty-eight hours following smoke exposure the animals were anesthetized with sodium pentobarbital, and paralyzed with pancuronium bromide (0.03 mg/kg IV, Organon Pharmaceuticals, West Orange, NJ), and bronchoalveolar lavage (BAL) was performed under mechanical ventilation. Twenty milliliters of 0.9% sterile saline was injected into the left lower lobe and the fluid was immediately aspirated. This process was repeated three times. The collected fluid was centrifuged and aliquots of the supernatant were stored at −70°C until measurement of total protein content. Total protein in the BAL fluid supernatant was measured using a spectrophotometric dye-binding protein assay (Bio-Rad).[17] The cell pellet was resuspended with the same volume of saline as the supernatant, and the total white blood cell count was determined using a hemocytometer. Differential cell counts were performed on Wright-Giemsa stained cytocentrifuge preparations.

After the animals were humanely killed, the wet-to-dry lung weight ratio (W/D) was determined by a modification of the gravimetric method of Drake et al.[18] The right lung was removed after the bronchi and vessels were ligated. The entire right lung was homogenized with an identical weight of distilled water. Duplicate samples of the homogenate and arterial blood were weighed and dried at 80°C. Dry weights were measured and the wet-to-dry ratios of the homogenate and blood were calculated. A sample of the homogenate was centrifuged at 14,500 rpm for 1 hour, and a blood sample was diluted with the same volume of distilled water. To determine the hemoglobin levels in the homogenate and blood, 20 μL of the homogenate supernatant or the diluted blood was added to 2.5 mL of Drabkin's solution. The absorbance of both solutions was measured spectrophotometrically at 540 nm. The blood weight in the wet lung was calculated. From these data, blood-free wet and dry weights of the right lung were calculated and the W/D ratio determined.

Conjugated diene levels in arterial plasma samples were measured by the method reported previously.[19] Briefly, 0.5 mL of plasma was mixed with 7.0 mL of a chloroform/methanol (2:1) dilution that had been preheated to 45°C. The

mixture was vortexed vigorously for 2 minutes, and then centrifuged for 5 minutes at 1500*g*. The lower chloroform layer was carefully removed and mixed with 2 mL of acidified water (pH 2.5). After the same agitation and centrifugation procedure was repeated, the lower level was aspirated and dried under nitrogen gas. The residue was reconstituted with 2 mL of heptane and the absorbance was measured spectrophotometrically at 233 nm. Results are expressed as absorbance at 233 nm against a blank consisting of heptane only.

Nitrate levels in arterial plasma samples were measured by a modification of the method of Green et al.[20]: 300 μL plasma was mixed with 150 μL 20% zinc sulfate solution and 150 μL 1 N sodium hydroxide. The mixture was vortexed and then allowed to stand for 5 minutes. After adding 10 mg silver lactate, the mixture was vortexed for 2 minutes and centrifuged at 13,000*g* for 5 minutes. Then 200 μL of the supernatant was pipetted to a glass tube, followed by 50 μL of internal standard (1 mmol/L9 4-nitro-o-xylene), and 500 μL sulfuric acid was added slowly and 200 μL benzene was quickly added. The tube was capped, vortexed for 2 minutes, and centrifuged at 2500*g* for 5 minutes. Approximately 170 μL of the upper benzene layer was transferred to a small tube containing 20 mg anhydrous sodium carbonate, vortexed for 15 seconds, and centrifuged at 2500*g* for 1 minute. Then 150 μL of the upper layer was transferred into an injection vial and total plasma nitrates were analyzed as nitrobenzene on a Hewlett-Packard 5890 Series 2 gas chromatograph employing a nitrogen-phosphorus thermionic detector. A standard curve using aqueous nitrate standards was constructed with results expressed as micromoles per liter.

Histologic Studies

Histologic evaluation of the tracheobronchial and parenchymal injury of each animal was performed using light microscopy and the following criteria.

Tracheobronchoepithelial Damage Score

0 = normal
1 = normal height of epithelium with some loss of cilia
2 = superficial erosion of epithelium with complete loss of cilia
3 = severe erosion of epithelium
4 = complete ulceration of epithelium

Lung Parenchymal Damage Score

0 = normal
1 = a few inflammatory cells in alveolar septa
2 = multifocal areas with increased inflammatory cells in alveolar septa or a few inflammatory cells in alveoli
3 = disseminated inflammation or edema or both in alveolar septa and alveoli that affect less than half the section
4 = diffuse inflammation or edema or both in alveolar septa and alveoli that affect more than half the section.

Statistical Analysis

Statistical analysis was performed using the Student's *t* test for comparisons between group 1 and group 2 at equivalent time points and analysis of variance (repeated measures) for comparisons of serial changes between the two groups. Analysis of variance was used to compare groups 1, 2, and 3 with post hoc testing using Tukey's method. Data are shown as mean ± standard error of the mean; significance was assigned at $p < 0.05$.

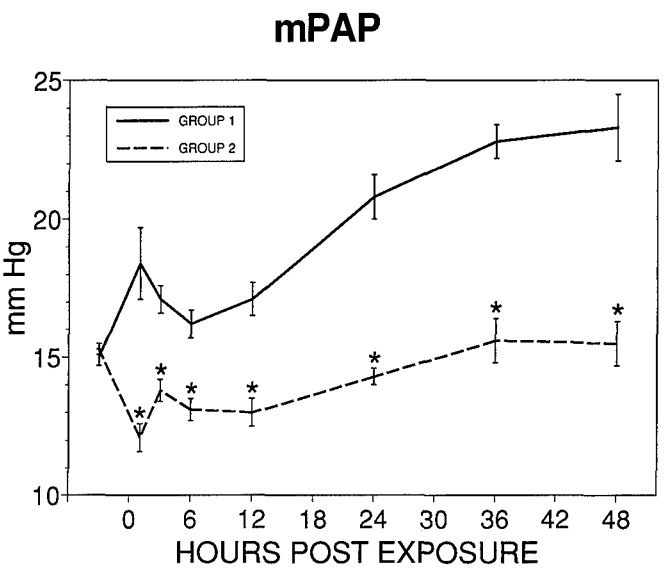

Figure 1. Serial mean pulmonary arterial pressures (mPAP) following smoke inhalation: Group 1 is represented by the solid line; group 2 by the dotted line; mPAP in group 2 was significantly lower than in group 1 throughout the study. (* $p < 0.05$, Student's *t* test at equivalent time).

RESULTS

All animals survived the 48-hour observation period. Arterial carboxyhemoglobin levels immediately after smoke exposure were 69.9% ± 3.5% in group 1 and 67.5% ± 4.9% in group 2 (p = N.S.). The nitrogen dioxide concentration of the inspired gas in group 2 was less than 1 ppm throughout the study.

Figure 1 depicts the serial mean pulmonary arterial pressure (MPAP) following smoke exposure. The MPAP was significantly higher in group 1 than group 2 throughout the study ($p < 0.05$).

Figure 2 depicts the serial pulmonary vascular resistance index (PVRI). The progressive elevation in PVRI

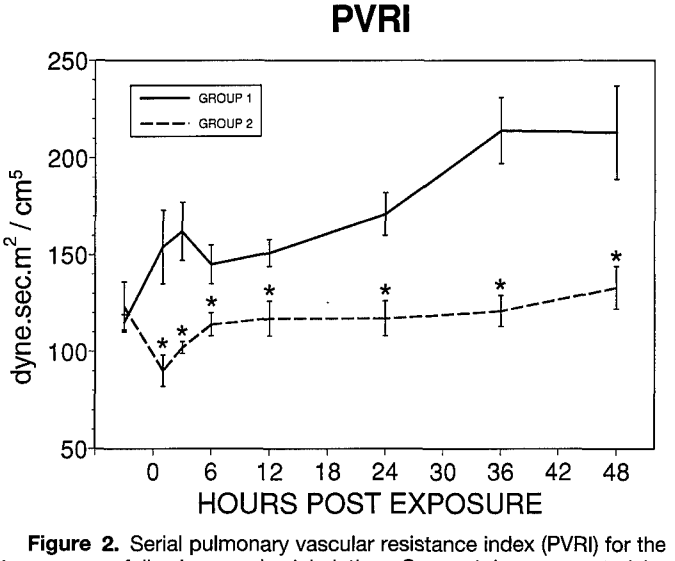

Figure 2. Serial pulmonary vascular resistance index (PVRI) for the two groups following smoke inhalation: Group 1 is represented by the solid line; group 2 by the dotted line; PVRI in group 2 was significantly lower than in group 1 throughout the study. (* $p < 0.05$, Student's *t* test at equivalent time).

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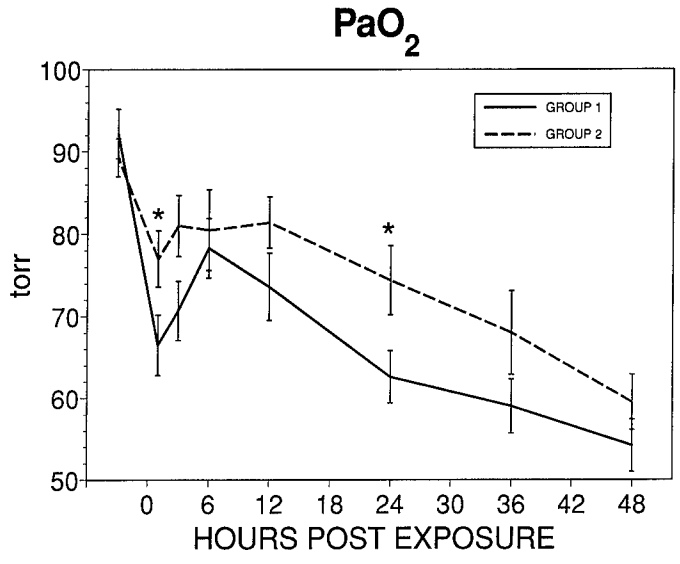

Figure 3. Serial Pao_2 following smoke inhalation: The mean value in group 2 was consistently higher than in group 1 throughout the study. The difference between the two groups was significant at the 1-hour and 24-hour time points. (* $p < 0.05$, Student's t test at equivalent time). The serial change pattern was significantly different between the two groups ($p < 0.05$, ANOVA repeated measures).

for group 1 was not found in group 2, and the difference between the two groups was significant throughout the study ($p < 0.05$).

Figure 3 shows the serial Pao_2 for the two groups. The mean Pao_2 in group 2 was consistently higher than in group 1 throughout the study. The difference between the two groups was significant at the 1-hour and 24-hour time points following smoke exposure. The serial change pattern was significantly different between the two groups ($p < 0.05$, ANOVA repeated measures).

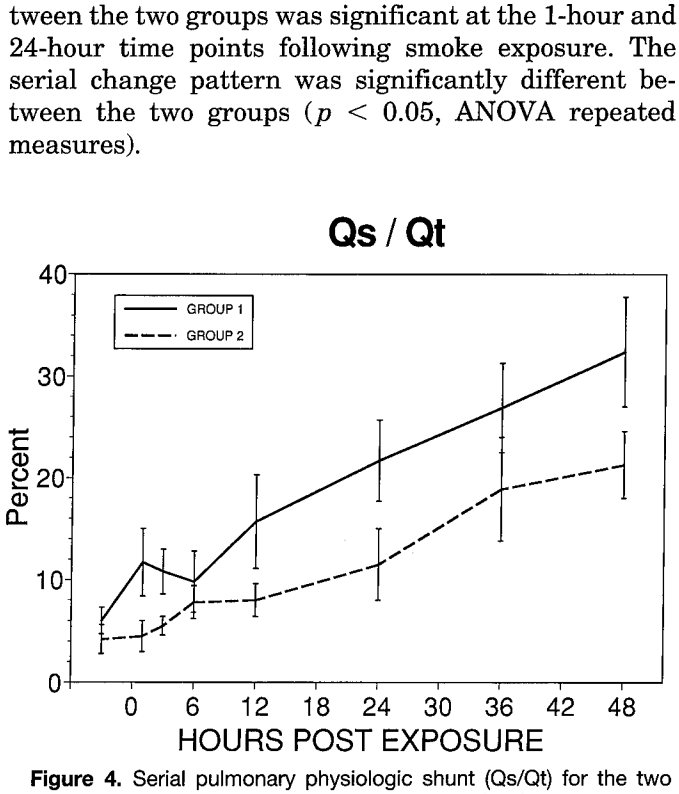

Figure 4. Serial pulmonary physiologic shunt (Qs/Qt) for the two groups following smoke inhalation: The mean value in group 2 was consistently smaller than in group 1 throughout the study. The serial change pattern was significantly different between the two groups ($p < 0.05$, ANOVA repeated measures).

Figure 4 shows the serial physiologic shunt (Qs/Qt). The mean Qs/Qt was consistently smaller in group 2 than in group 1 throughout the study and the serial change pattern was significantly different between the two groups ($p < 0.05$, ANOVA repeated measures).

Table 1 shows other cardiopulmonary variables. Pulmonary capillary wedge pressure was significantly higher in group 1 than in group 2 during the second 24 hours of the study. The mean respiratory index (RI) was consistently higher in group 1 compared with group 2. The serial change pattern of RI was significantly different between the two groups (ANOVA repeated measures). The $Paco_2$, mean systemic arterial pressure, total peripheral resistance index, cardiac index, oxygen delivery (Do_2), and oxygen consumption (Vo_2) did not differ significantly between the two groups throughout the study.

The recovery of BAL fluid in this study was approximately 60%. Figure 5 shows the total white blood cell (WBC) and polymorphonuclear leukocyte (PMN) counts in BAL fluid from groups 1, 2, and 3 (animal controls). Both numbers were significantly higher in groups 1 and 2 compared with normal controls ($p < 0.05$, ANOVA Tukey), but the difference between groups 1 and 2 was not statistically significant.

Table 2 depicts the total protein content in BAL fluid and the wet-to-dry lung weight ratios (W/D) for groups 1, 2, and 3. Both values for groups 1 and 2 were significantly higher than in the normal group. There was no significant difference in total protein content in BAL fluid and W/D between groups 1 and 2.

Figure 6 depicts the changes in static lung compliance and pulmonary resistance at 48 hours compared with the pre-exposure baseline values for groups 1 and 2. The changes in both variables were not statistically different between the two groups.

Table 3 shows arterial conjugated diene levels for the two groups. There was no significant difference between the groups following smoke exposure. Figure 7 depicts the serial arterial nitrate levels. The level was consistently higher in group 2 than in group 1, and the difference between the two groups was significant at the 3-hour point and for the duration of the study ($p < 0.05$).

Table 4 contains serial arterial methemoglobin and carboxyhemoglobin levels, and platelet counts. Methemoglobin in group 2 was significantly higher than in group 1 at 3 hours and for the duration of the study ($p < 0.05$). There were no significant differences in carboxyhemoglobin levels. Platelet counts gradually decreased in both groups, but the difference between the two groups was not significant.

Table 5 contains the histologic scores at the level of mid trachea, left main bronchus, segmental bronchus, and left lower lobe parenchyma. The damage scores at each level did not differ significantly between groups 1 and 2.

Table 1
Other cardiopulmonary variables

	Baseline	Hours					
		3	6	12	24	36	48
PCWP (mm Hg)							
Group 1	8.6 ± 0.4	7.9 ± 0.4	8.1 ± 0.4	8.3 ± 0.6	9.0 ± 0.4	9.8 ± 0.6	10.0 ± 0.5
Group 2	8.0 ± 0.5	7.6 ± 0.3	7.1 ± 0.4	6.9 ± 0.3	7.8 ± 0.3*	7.9 ± 0.4*	7.9 ± 0.4*
RI							
Group 1	0.05 ± 0.02	0.33 ± 0.06	0.24 ± 0.06	0.32 ± 0.08	0.55 ± 0.08	0.62 ± 0.08	0.68 ± 0.07
Group 2	0.07 ± 0.02	0.17 ± 0.05	0.22 ± 0.07	0.18 ± 0.04	0.29 ± 0.08*	0.40 ± 0.07	0.59 ± 0.08
$Paco_2$							
Group 1	30.8 ± 1.0	32.7 ± 0.8	29.7 ± 1.0	30.2 ± 1.3	30.2 ± 1.8	31.2 ± 1.1	35.1 ± 2.7
Group 2	31.2 ± 1.0	31.5 ± 0.9	29.5 ± 1.0	30.1 ± 1.1	31.2 ± 1.0	32.2 ± 2.1	32.2 ± 1.9
mSAP (mm Hg)							
Group 1	93.8 ± 3.3	95.6 ± 2.7	93.8 ± 2.1	92.3 ± 3.3	93.7 ± 4.4	96.8 ± 3.4	101.9 ± 4.6
Group 2	92.1 ± 3.4	92.3 ± 3.4	95.3 ± 3.6	92.9 ± 3.8	96.5 ± 5.0	103.9 ± 6.0	101.9 ± 5.7
TPRI (dyne · sec · m^2)							
Group 1	1630 ± 57	1665 ± 143	1635 ± 78	1539 ± 66	1393 ± 74	1544 ± 122	1535 ± 117
Group 2	1506 ± 62	1529 ± 101	1787 ± 99	1739 ± 122	1718 ± 135	1715 ± 223	1736 ± 89
CI (L/min/m^2)							
Group 1	4.5 ± 0.1	4.8 ± 0.4	4.6 ± 0.2	4.7 ± 0.3	5.1 ± 0.2	5.0 ± 0.3	5.2 ± 0.3
Group 2	4.9 ± 0.3	4.8 ± 0.2	4.2 ± 0.2	4.2 ± 0.2	4.5 ± 0.2	5.1 ± 0.5	4.6 ± 0.2
DO_2/BSA (mL/min/m^2)							
Group 1	663 ± 36	499 ± 39	540 ± 27	569 ± 24	635 ± 34	600 ± 28	570 ± 47
Group 2	712 ± 47	521 ± 30	539 ± 24	525 ± 17	564 ± 30	607 ± 56	551 ± 30
VO_2/BSA (mL/min/m^2)							
Group 1	201 ± 5	162 ± 14	187 ± 12	194 ± 12	221 ± 9	199 ± 16	199 ± 13
Group 2	182 ± 7	169 ± 9	184 ± 5	176 ± 6	199 ± 14	209 ± 25	201 ± 13

PCWP = pulmonary capillary wedge pressure; RI = respiratory index; mSAP = mean systemic arterial pressure; TPRI = total peripheral resistance index; CI = cardiac index; DO_2/BSA = oxygen delivery index; BSA (m^2) = total body surface area; VO_2/BSA = oxygen consumption index. Values are means ± SEM.

* $p < 0.05$, Student's t test at equivalent time.

DISCUSSION

Noxious chemicals generated from incomplete combustion induce direct injury to exposed airways, triggering the production of inflammatory mediators and the accumulation of activated leukocytes in the lung.[21] Polymorphonuclear leukocytes (PMNs) may play a significant role in the progressive inflammatory process that follows smoke exposure.[22] Airway inflammation and subsequent edema formation secondary to increased pulmonary capillary pressure and permeability readily occlude the small airways and alveolar space, increasing true shunt and low ventilation-perfusion (VA/Q) areas in the lung. Vasodilatation of bronchial blood vessels and pulmonary vasoconstriction have been reported to occur simultaneously secondary to the increased production of neuropeptides, thromboxanes, leukotrienes, and cytokines such as PAF, as well as from increased VA/Q mismatching and hypoxemia.[2,23–26] The pathophysiologic effects of pulmonary vasoconstriction following smoke exposure have not been clarified.

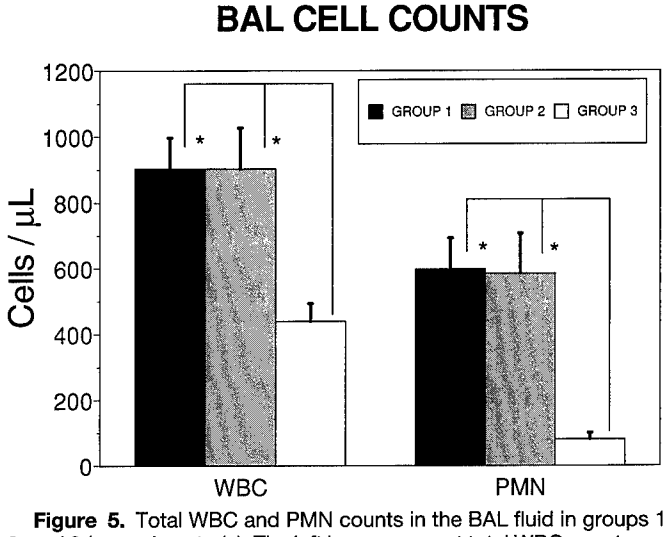

Figure 5. Total WBC and PMN counts in the BAL fluid in groups 1, 2, and 3 (normal controls): The left bars represent total WBC counts and the right bars represent PMN counts. Both values in groups 1 and 2 were significantly higher than in group 3, but the difference between groups 1 and 2 was not significant. (* $p < 0.05$, ANOVA Tukey).

Table 2
Total protein content in BAL fluid: Wet-to-dry lung weight ratio (W/D)

	Total Protein in BAL Fluid (µg/mL)	W/D
Normal	251 ± 46	4.27 ± 0.09
Group 1	523 ± 118*	5.76 ± 0.33*
Group 2	577 ± 126*	5.76 ± 0.33*

Values are means ± SEM.

* $p < 0.05$ versus normal (ANOVA Tukey).

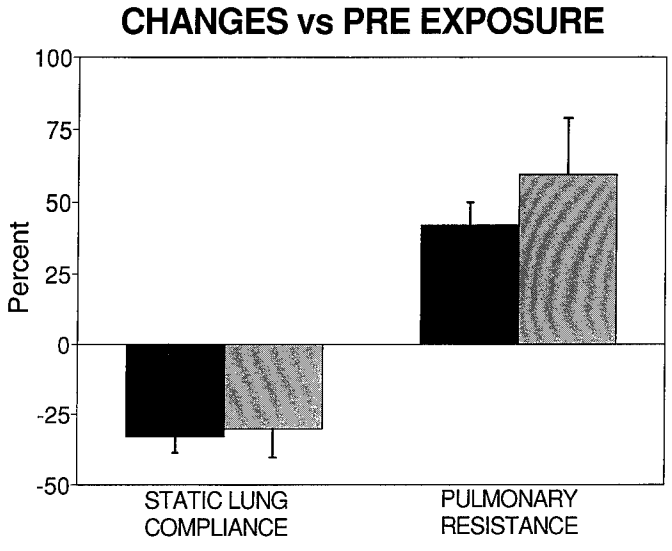

Figure 6. Changes in static lung compliance and pulmonary resistance at 48 hours compared with baseline pre-exposure values: The changes in both variables did not differ significantly between groups 1 and 2.

Endogenous nitric oxide is formed by the oxidation of the terminal guanidino nitrogen of L-arginine, which is catalyzed by nitric oxide synthase. Nitric oxide activates soluble guanylate cyclase, which increases intracellular cGMP and decreases free cytosolic calcium levels.[4–6,27] Nitric oxide also appears to inhibit the production and action of endothelin.[28] These actions induce relaxation of vascular and tracheal smooth muscle cells.[29] In vitro, nitric oxide inhibits platelet adhesion to cultured endothelial cells, and inhibition of neutrophil adhesion to mesenteric venules by nitric oxide has been observed in vivo.[7,30] A cytoprotective effect of nitric oxide has been reported in animal models following ischemia-reperfusion in splanchnic, coronary, and cerebral arterial beds.[31–33] Nitric oxide also participates in inflammatory and autoimmune-mediated tissue injury.[8] Deoxyribonucleic acid damage and mutation have been reported to occur in human cells exposed to nitric oxide in vitro.[34] Finally, overproduction of nitric oxide appears to be involved in the hemodynamic alterations that occur in septic shock.[35]

Recently, low concentrations of inhaled nitric oxide (5–150 ppm) have been used as a selective pulmonary vasodilator. Inhaled nitric oxide is delivered deep into the lung parenchyma because of its lipophilic nature and low solubility in water, and readily permeates the cell membrane and binds intracellular guanylate cyclase in pulmonary vascular smooth muscle cells. When absorbed into the blood stream, nitric oxide reacts with great affinity with oxyhemoglobin to form nitrosyl hemoglobin, which is then oxidized to methemoglobin with production of nitrite and nitrate, which are then excreted into the urine.[36] Within the respiratory circuit and lung nitric oxide is also gradually oxidized to nitrogen dioxide depending on the oxygen and nitric oxide concentrations as well as contact time. Nitrogen oxides, especially nitrogen dioxide, have been considered to be harmful when inhaled. Exposure to concentrations greater than 50 ppm of nitrogen dioxide for 5 minutes induces significant lung injury in rats, but 1000 ppm of nitric oxide for 30 minutes did not induce histologic pulmonary change.[37]

In animal studies, inhaled nitric oxide has been shown to attenuate pulmonary vasoconstriction following hypoxemia, exogenous administration of thromboxane analogs, heparin/protamine therapy, and sepsis,[9–11] whereas 80 ppm of inhaled nitric oxide had no pulmonary vascular effects in normal sheep. Inhaled nitric oxide has also been shown to attenuate elevated pulmonary vascular resistance in patients with chronic pulmonary arterial hypertension, persistent pulmonary hypertension of the newborn, ARDS, congenital heart failure, obstructive lung disease, and pneumonia.[12–16,38] Eighteen to 36 ppm of inhaled nitric oxide significantly improved gas exchange in ARDS patients by improving pulmonary VA/Q matching; gas exchange was not improved by the intravenous administration of prostacyclin. These findings highlight the specific advantage of the selective vasodilatation of ventilated areas by nitric oxide.[13] In the same study, 5 to 20 ppm of inhaled nitric oxide administered for 3 to 53 days had no adverse effects in a small group of ARDS patients.

Table 3
Arterial conjugated dienes

	Baseline	24 Hours	48 Hours
Group 1	0.88 ± 0.07	1.04 ± 0.10	0.94 ± 0.07
Group 2	0.83 ± 0.06	0.94 ± 0.06	0.95 ± 0.07

Values are expressed as absorbance at 233 nm (means ± SEM).

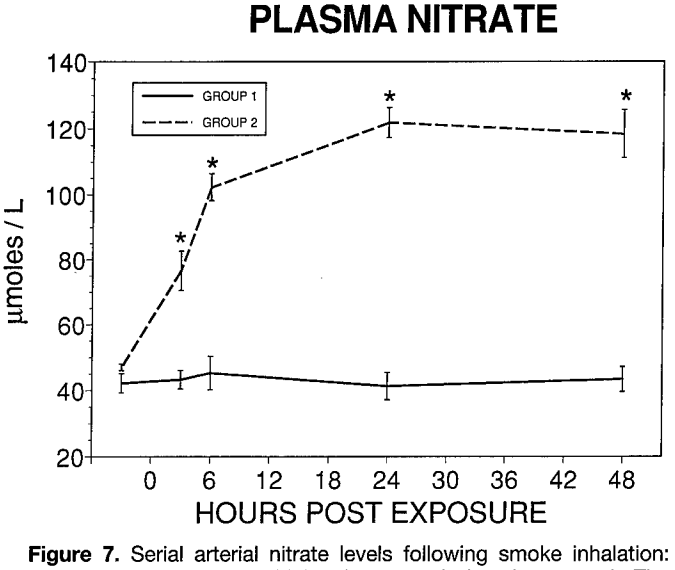

Figure 7. Serial arterial nitrate levels following smoke inhalation: The levels are consistently higher in group 2 than in group 1. The difference between the two groups was significant at the 3-hour point and for the duration of the study. (* $p < 0.05$, Student's t test at equivalent time).

Table 4
Arterial methemoglobin and carboxyhemoglobin levels and platelet counts

	Baseline	Hours						
		1	3	6	12	24	36	48
Met-Hb (%)								
Group 1	1.4 ± 0.1	1.4 ± 0.0	1.5 ± 0.0	1.4 ± 0.0	1.4 ± 0.1	1.3 ± 0.0	1.4 ± 0.1	1.3 ± 0.1
Group 2	1.3 ± 0.1	1.6 ± 0.1	2.0 ± 0.1*	2.0 ± 0.2*	2.0 ± 0.2*	2.0 ± 0.1*	2.0 ± 0.2*	1.9 ± 0.2*
CO-Hb (%)								
Group 1	5.9 ± 0.3	36.2 ± 2.2	23.0 ± 1.6	12.5 ± 0.9	7.3 ± 0.4	5.5 ± 0.3	5.3 ± 0.3	5.1 ± 0.5
Group 2	6.2 ± 0.2	33.7 ± 2.4	21.5 ± 1.5	12.6 ± 1.0	7.2 ± 0.4	5.7 ± 0.3	5.2 ± 0.4	5.4 ± 0.3
PLT (×1000/μL)								
Group 1	459 ± 56	378 ± 39	346 ± 32	314 ± 25	280 ± 24	233 ± 26	149 ± 26	140 ± 21
Group 2	443 ± 66	363 ± 29	343 ± 26	343 ± 25	302 ± 26	230 ± 14	214 ± 13	183 ± 18

Values are means ± SEM.
* $p < 0.05$, Student's t test at equivalent time.

In the present study, pulmonary arterial hypertension was significantly attenuated by inhaled nitric oxide following smoke exposure. This effect was significant within 30 minutes of initiation of therapy and persisted throughout the study. The increased arterial methemoglobin and plasma nitrate levels, as well as the lack of systemic circulatory effects, are consistent with rapid inactivation or metabolism of nitric oxide. The increased arterial methemoglobin levels in the treated group produced no apparent adverse effects. The plasma nitrate levels were significantly elevated following inhaled nitric oxide, indicating its permeation from the respiratory tract and alveoli into the vessels during treatment.

The decrement in pulmonary oxygenation documented following smoke exposure was lessened by inhaled nitric oxide. This is consistent with a smaller physiologic shunt in the treated group. Smoke inhalation has been reported to increase low VA/Q areas in the lung, and inhaled nitric oxide may increase blood flow to the better ventilated areas. No bronchodilatory effect was observed, possibly because of obstructive pseudomembrane formation following smoke exposure; pulmonary resistance and P_{CO_2} did not change following therapy.

In both groups, smoke exposure resulted in loss of cilia, erosion and sloughing of bronchoepithelial cells, and pseudomembrane formation in the airway, and disseminated inflammation and edema were observed in the alveolar septi and alveoli. Inhaled nitric oxide did not alter these histologic changes, suggesting that inhaled nitric oxide did not affect the inflammatory process that occurs following smoke exposure. The inhibitory effect of nitric oxide on neutrophil adhesion to pulmonary vascular endothelium was not apparent. Nitric oxide and its metabolites did not increase the severity of the inhalation injury. This is consistent with other reports in which increasing the blood flow to smoke-exposed lung by mechanical techniques or dopamine therapy has been shown not to affect injury severity.[39,40] During the 48-hour observation period no significant adverse cardiopulmonary effects were observed.

Table 5
Histologic damage scores by light microscopy

	Mid Trachea	Main Bronchus	Segmental Bronchus	Lower Lobe Parenchyma
Normal	0.0 ± 0.0	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2
Group 1	2.6 ± 0.4*	2.8 ± 0.5*	2.8 ± 0.4*	2.4 ± 0.2*
Group 2	2.4 ± 0.3*	2.8 ± 0.4*	2.8 ± 0.4*	2.6 ± 0.3*

Criteria for damage scores are shown in Methods and Materials. Values are means ± SEM.
* $p < 0.05$ versus normal (ANOVA Tukey).

Although inhaled nitric oxide lowered mean pulmonary arterial pressure and pulmonary capillary wedge pressure and attenuated pulmonary capillary hypertension, extravascular lung water and total protein content of the BAL fluid did not change, indicating that inhaled nitric oxide had no effect on the increased pulmonary capillary permeability that follows smoke inhalation.

In summary, inhaled nitric oxide significantly attenuated pulmonary arterial hypertension in an animal model of inhalation injury and was associated with no apparent toxicity. This effect on pulmonary hemodynamics was accompanied by a modest improvement in oxygenation, but treatment with nitric oxide did not alter either the tracheobronchial and alveolar lesions or the increase in lung water observed in these animals. The attenuation of deleterious pulmonary physiologic changes in the absence of morphologic improvement emphasizes the importance of redistribution of pulmonary blood flow in both the vascular effects of inhalation injury and the clinical support of patients with this form of pulmonary insult. The utilization of inhaled nitric oxide in patients with inhalation injury should permit combination therapy with an agent that can ameliorate the effects of inflammatory mediators, as well as use of ventilatory support systems employing lower airway pressures, which will reduce the risk of barotrauma.

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DISCUSSION

Dr. Robert H. Demling (Boston, Massachusetts): I congratulate Doctor Ogura and his group on a very fine study. As you can see, nitric oxide is sort of a double-edged sword in that there are as many potentially deleterious as potentially therapeutic properties of this particular agent. And I think the key piece of information of most importance in this study is the fact that nitric oxide does not appear to be a causative factor in the early bronchorrhea and bronchial injury caused by inhalation injury, since it has been high on the list of potential causes for a long time. And for that purpose, I think this study has a major potential for significant scientific evidence as far as our ability to unravel what happens with early inhalation injury. The authors also pointed out that there may be some potential early therapeutic benefit of using nitric oxide for its vasodilator properties, assuming that the early vasoconstriction is mediator induced.

One concern in this particular area is, as you know, that nitric oxide has to get to the capillaries. As the disease progresses, as was noted, with the progressive shunt fraction over time the potential of the nitric oxide getting out to the capillaries becomes less and less.

So one comment I would like the authors to discuss would be: Is this actually going to be a short-term therapeutic modality given the fact that as airways continue to close over time in a standard inhalation injury, the ability to get the nitric oxide out to the capillaries will decrease?

The second area of interest and importance as far as follow-up studies would be the fact that inhalation injury, as you well know, changes over time in terms of its causes and pathologic factors. Early on the primary problem is mechanical airway obstruction from edema, and this is followed by a generalized inflammatory lung where a number of mediators perpetuate the disease process. And it is very likely and possible that nitric oxide, although being innocuous in the early aspects of the disease state, actually becomes a perpetuator of the disease process later on because inflammation becomes a more important component of the disease beginning at about 48 to 72 hours.

The second question I would like to ask the authors therefore is: Are they planning on pursuing the time course, since they have a very nice model of inhalation injury, to see whether in fact nitric oxide at a later date actually becomes a deleterious agent rather than a therapeutic agent? Thank you very much.

Dr. Andrew B. Peitzman (Pittsburgh, Pennsylvania): We have used inhaled nitric oxide in a pig model of acute lung injury using oleic acid. These data were presented at the Surgical Infection Society earlier this year. Our findings were very similar to yours using inhaled nitric oxide. We saw an increase in Pao_2, decrease in shunt, and decrease in pulmonary vascular resistance. But, similarly, this occurred without amelioration of the histologic changes or the inflammatory changes that were described. I have two questions for the authors.

First, why did you choose 20 parts per million of inhaled nitric oxide? Have you done studies using higher concentrations?

Second, inhaled nitric oxide has been used clinically in infants and adults to treat acute respiratory distress syndrome. Do you have plans to use inhaled nitric oxide clinically in the treatment of patients with inhalation injury?

Dr. David J. Dries (Maywood, Illinois): I have two questions. I would like to turn one of the questions that Doctor Demling asked around and ask the authors if they think that there may be a prophylactic role for early administration of nitric oxide where inhalation injury is suspected.

I was also wondering if the authors could comment, since they have so much experience with inhalation injury, what component does right ventricular failure play in the end-stage response to this disease and do they think that the improvement in the pulmonary hemodynamics seen with nitric oxide administration may actually play a role in ameliorating right ventricular failure? Thank you.

Dr. Hiroshi Ogura (Closing): We thank Doctor Demling and the other discussants. In response to Dr. Demling's first question, we have recently completed another study of the effects of inhaled nitric oxide following smoke inhalation using a multiple inert gas elimination technique in mechanically ventilated animals. In that study, inhaled nitric oxide significantly improved pulmonary oxygenation by decreasing the fraction of blood flow in the shunt and low VA/Q areas; this appears to have been due to specific vasodilatation in the ventilated areas of the lung and not a bronchodilator affect. Since the beneficial effects of inhaled nitric oxide result from dilatation of small arteries in the well-ventilated areas, closure of these airways might blunt the effect, but one must remember that the effect we have observed occurs in the presence of some airway closure. As to the second question, we did not test a longer period of treatment. In this model severe progressive airway and pulmonary inflammation occur during the 48 hours following injury, and in that interval, at least, we saw indication of toxicity.

In response to Doctor Peitzman, previous animal studies have used concentrations ranging from 5 to 150 parts per million. These concentrations all significantly inhibited pulmonary arterial hypertension following a variety of pulmonary insults. Since nitric oxide is gradually metabolized to more noxious nitrogen dioxide we felt we should use a relatively low concentration, and selected 20ppm after preliminary tests of several concentrations. Our recent study also indicates that inhaled NO siginificantly improves pulmonary function in experimental sepsis, suggesting that inhaled NO could find clinical application in both inhalation injury and sepsis associated with such injury. At present, however, our information does not warrant clinical trial.

In answer to Doctor Dries, inhaled nitric oxide modestly improved pulmonary oxygenation without affecting the morphologic changes consequent to smoke inhalation. Combined treatment with NO and anti-inflammatory agents nitric oxide attenuates pulmonary hypertension and might potentially improve the right ventricular ejection fraction, we did not evaluate right ventricular function in the present study. Thank you very much.